

THE X-IRRADIATION OF MARINE GAMETES.¹ A STUDY OF
THE EFFECTS OF X-IRRADIATION AT DIFFERENT
LEVELS ON THE GERM CELLS OF THE CLAM,
SPISULA (FORMERLY MACTRA)

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This study was made for the following reasons:

1. To determine the radiosensitivity of the sperm and eggs of the clam and to compare it with the radiosensitivity of *Arbacia* gametes.
2. To determine whether cysteine or the new Bacq solution (B-mercaptoethylamine) has any protective effect on the gametes and early development of marine forms.
3. To determine whether activation and syngamy could be separated by x-irradiation, and, if so,
4. To determine whether cross-fertilization with irradiated gametes and intergeneric parthenogenesis could be accomplished.

MATERIALS AND METHOD

The eggs and sperm of the mollusc, *Spisula solidissima*, were used in these studies.

The eggs of the clam *Spisula* seem to be produced almost continuously, and (according to Allen) are available except for a period of about 10 days in August (Allen, 1951). The shell is cracked along the two-year line and the ovaries are cut out and dissected into small pieces on cheese-cloth suspended in a beaker of filtered sea water. The eggs pass through the cheese-cloth leaving behind the ovarian tissue. The ovarian masses are gently agitated to liberate the eggs. About one hour later, the sea water is decanted off and fresh (filtered) sea water is added in sufficient quantity to bathe thoroughly the eggs. This process is repeated after the eggs have settled so that the eggs get at least two thorough washings. After this they can be fertilized, or can be set aside at room temperature for use within 6-8 hours, or can be placed in the refrigerator at 10 degrees C. for use during the next 24-30 hours. The fertilization after 24 hours is still 100%, but drops to about 40% in 48 hours. Eggs in refrigeration are brought to room temperature and changed to fresh sea water before use.

The sperm are obtained by cutting the testes into small pieces in a minimum of sea water in No. 2 Stender dishes. These so-called "dry sperm" may be kept in covered dishes for dilution and use during 24 hours, or, if placed in refrigeration at 10 degrees C. may be kept for 48 hours longer. Just before use in fertilization,

¹This document is based on work performed under Contract AT-30-1-GEN-70 for the Atomic Energy Commission.

one drop of dry sperm is added to 40 cc. of sea water (No. 2 Stender-full) and the suspension made homogeneous with pipette stirring. Then one pipette-full of this suspension is added to a finger bowl of eggs to secure normal fertilization of all eggs. If the sperm are refrigerated, they are given about ten minutes in suspension to become thoroughly activated. In all experiments involving irradiated sperm similar dilutions were made up after irradiation and similar amounts of diluted spermatozoa were used.

The two presumably protective chemicals used were cysteine hydrochloride and another —SH solution obtained from Dr. Bacq of Bruges, known as 1573L. It is B-mercaptoethylamine. Dr. Bacq has demonstrated (1951) the protective value of this drug for mice, and claims that it is even more effective than is cysteine. The cysteine experiments have been carried on largely at the Argonne Laboratories by Patt and his co-workers (1949, 1950).

The solution obtained from Dr. Bacq is in ampoules of 10 cc. each representing 100 mg., and has been demonstrated by him to be non-toxic but protective to mice against x-irradiation when used at levels of three mg. per mouse of average weight of 30 grams (Bacq *et al.*, 1951). The undiluted solution has a pH of approximately 7.5. It was diluted in filtered sea water and adjusted to a pH of 8.0. The concentrations used were designated in milligrams per cent. It was found that 0.5 mg. % was lethal to *Arbacia plutei* (used as test material) and that 0.01 mg. % was non-toxic to gametes and early embryos. Twice this concentration, 0.02 mg. %, was toxic for *Arbacia plutei* over a 24-hour period.

Cysteine hydrochloride was obtained in the crystalline form and it was found that a 1% solution in sea water had a pH of 1.9. This solution was made up fresh for each experiment. It was adjusted with concentrated NaOH to a pH of approximately 8 which is in the range of normal sea water. X-irradiation to 18,900 r did not alter the pH of the cysteine solution. Cysteine in solution is more stable as an acid and unless kept free from air (oxygen) and at refrigeration temperatures, it develops a flocculent sediment due to degeneration to cystine. It is known that cysteine activates enzymes, combines with H_2O_2 (which is known to be formed in irradiation solutions) and rapidly oxidizes to the non-protective SS-cystine. It was found that a 0.5% solution caused a slight shrinking of clam eggs and a wrinkling of the surface, suggestive of hypertonicity. A 0.1% solution had no such effect and cleavage to 100% could be obtained. However, development was abnormal, so that a solution of 0.01% was used for these experiments.

In all fertilization experiments controls were set up at the beginning and at the end of each series so that the time factor of the experiment was not involved. Regulation finger bowls were used, each of which had been pre-tested for possible contamination. This was done by using sea water and living *Arbacia* embryos kept over a period of three days. Each bowl contained 100 cc. of filtered sea water, and all bowls were kept under the same conditions of light and temperature.

The x-ray facilities were those of the Marine Biological Laboratory, Woods Hole, Mass. The factors were 182 kv, 25 ma with two alternate parallel tubes having equivalent filtration of 0.2 mm. Cu and output of 6300 r/min., under conditions used in these experiments. When eggs or sperm were to be irradiated for more than four minutes, the plastic fly box in which they were placed was surrounded by ice cubes so that heat emanating from the filaments of the x-ray tubes

would not affect the material. The plastic containers had been previously tested against living embryos for toxicity.

OBSERVATION AND EXPERIMENTAL DATA

Spisula

Spermatozoa

The spermatozoa of this genus are more radioresistant than are the eggs. This was determined by studying the effect of x-irradiated sperm on cleavage and on early development when used with normal, unirradiated eggs. The results were compared with those obtained when normal sperm were used with x-irradiated eggs.

Fertilization of normal eggs was accomplished to approximately 100% with concentrated (dry) spermatozoa exposed to all levels of x-irradiation from 3,150 r to 264,600 r. One can say, therefore, that the mechanics of fertilization seem to be in no way altered by the x-irradiation of spermatozoa of this species at any level studied.

Fertilizing power in all vertebrates naturally presumes motility on the part of the spermatozoa. However, motility alone is no guarantee of successful fertilization. Motility was not stopped in concentrated sperm irradiated to the maximum level of 264,600 r and then diluted. If the highly exposed spermatozoa are kept concentrated and left at room temperature (23–25 degrees C.) for 24 hours, only about 10% of the eggs are fertilized, and very few of these develop into motile trochophores. An exposure of concentrated sperm to 189,000 r followed by 24 hours in the refrigerator at 10 degrees C., did not alter the motility or the fertilizing power of *Spisula* spermatozoa. Many of the trochophores produced from such sperm and normal eggs appeared to be quite normal. With further refrigeration to 48 hours there was complete loss of fertilizing power by the spermatozoa. Controls, under similar conditions, gave normal fertilization and normal trochophores.

Cleavage delay of normal eggs fertilized by irradiated sperm was slightly increased with increasing exposures to x-irradiation.

Table I represents the average of six sets of readings, each of which involved thousands of eggs. Experiments were performed at the laboratory temperature of 23–25 degrees C.

Table I points up two possibilities. (1) The interval between the time of fertilization and the time of first cleavage of 50% of the eggs is increased by x-irradiation of the spermatozoa alone, but the increase is not linear nor does it follow any ratio with respect to the increased irradiation. The delay is increased to a maximum at about 113,400 r and then the curve shows a plateau of effect or even a slight decrease with further irradiation. This suggests that while the activating factors of the spermatozoon are not altered (*i.e.*, fertilization is achieved in all), the genetic material of the sperm, the syngamic or even the mitotic equipment, is so damaged that the initial cleavage is altered. The maximum delay of 15 minutes represents approximately the normal interval between the first and the second cleavages, so that possibly the first cleavage is actually omitted at 163,800 r or above. To substantiate this thesis further it might be pointed out that after the plateau is reached, there is never any reduction of the cleavage delay to a level which might be compared with the controls. Haploid (parthenogenetic) embryos have been produced among

other genera, and by other means, which showed such cleavage delay and abnormalities (Fankhauser, 1945; Parmenter, 1933, 1940; Harvey, 1936, 1940; Tyler, 1941; Tyler and Bauer, 1937; Rostand, 1938; Porter, 1939; Kawamura, 1939; Tchou-Su and Chen-Chou-Hsi, 1940). It should be emphasized further that cleavage of 50% of the eggs includes all eggs fertilized, and some of these may have been fertilized by spermatozoa of varying radiation damage so that the time lapse represents an average of the functional activity of all damaged sperm. It is likely that in these eggs there is an internal maladjustment of normal chromosomal material from the egg with variously damaged chromosomal material from the x-irradiated sperm (assuming that no two sperm were necessarily damaged in the same way). In some eggs the partially damaged sperm genetic material may have little adverse effect while in others it may be completely incompatible with the normal egg complement and thereby either kill the zygote or cause developmental abnor-

TABLE I

Sperm condition	Time from fertilization to first cleavage for 50% of eggs	Range of times	Delay in minutes	Trochophore %
Sea water control	58"	56- 61	0	100
6,300 r	1'02"	59-1'04"	4	100
12,600 r	1'03"	01-1'05"	4	100
18,900 r	1'05"	1'03-1'06"	7	72
25,200 r	1'07"	1'05-1'08"	9	45
31,500 r	1'09"	1'05-1'14"	11	62
44,100 r	1'10"	1'08-1'13"	12	55
63,000 r	1'11"	1'07-1'16"	13	48
89,200 r	1'13"	1'07-1'17"	15	60
113,400 r	1'12"	1'06-1'17"	14	45
138,600 r	1'13"	1'07-1'20"	15	40
163,800 r	1'10"	1'04-1'15"	12	33
170,400 r	1'09"	1'05-1'14"	11	40
189,000 r	1'10"	1'08-1'11"	12	65
214,200 r	1'12"	1'06-1'16"	14	85
239,400 r	1'11"	1'06-1'15"	13	95
264,600 r	1'10"	1'06-1'15"	12	95

malities (O. Hertwig, 1910, 1911; P. Hertwig, 1911, 1916; Rugh, 1939a, 1939b). Further (2) if one studies the trochophores (early embryos) arising from these fertilized eggs one finds that the low 6,300 r level of exposure as well as the very high 264,600 r exposure each produces nearly as many motile, ciliated trochophores as did the control sperm. The low point of trochophore production and maximum incidence of abnormalities is at about 163,800 r, suggesting that this level of exposure of spermatozoa causes the maximum of developmental incompatibilities. The abnormal trochophores also exhibit a wide range of sizes. Fertilization incompatibilities are overcome by further irradiation of the spermatozoa (Rugh and Exner, 1940). It is true that the trochophores from highly irradiated sperm are not normal and that they do not develop beyond 30 hours, but they are structurally quite uniform and appear to be very much like those of the controls. This may be due to the probability that these are haploid embryos. This further substantiates the thesis

that the greater the irradiation of the sperm the less deleterious effect will the sperm genetic material have on the developmental process of the resulting zygote and embryo.

Since concentrated spermatozoa can be exposed to 264,600 r and, if properly diluted, can still fertilize 100% of the available eggs and since these in turn become ciliated and motile trochophores within 6 hours at laboratory temperatures, it seems to be difficult if not impossible to obliterate or alter the fertilizing mechanism of the spermatozoa by x-irradiation. It is presumably only the genetic (chromosomal) material that is damaged and this occurs at the lower levels of irradiation. This is not evident, however, until the developmental processes are brought into play.

All the above observations were based on the x-irradiation of dry (concentrated) spermatozoa. It was obviously in order similarly to irradiate sperm at

TABLE II
*Fertilization percentage of normal eggs and irradiated sperm**

Exposure	Dilutions of Sperm**		
	1/20	1/200	1/2000
Controls	100	100	100
6,300 r	95	95	90
9,450 r	95	95	80
12,600 r	95	80	50
15,750 r	95	75	30
18,900 r	95	60	30
22,050 r	90	45	20
25,200 r	95	33	0
28,350 r	90	5	0
31,500 r	95	1	0
Controls***	90	95	80

* Values are per cent cleavage in all eggs studied.

** The number of sperm for each batch of eggs could not be controlled accurately. Nevertheless, the same method of sampling was used throughout so that it can be assumed that the numbers of available sperm were rather constant.

*** Since the process of irradiation involved time, a second set of non-irradiated controls was used at the termination of the irradiation period. In this way the time factor above could be included.

various dilutions to compare the radiosensitivity. Here the situation is quite different.

Concentrated (dry) sperm may be used for many hours, particularly if they are kept in the refrigerator in a covered dish to prevent desiccation. The sperm dilution experiments, to the contrary, had to be conducted within a relatively short time since it is well known that mere dilution will activate spermatozoa which have a limited reserve of energy and consequently a limited life (Lillie, 1919). One cubic centimeter of fluid may be roughly considered as 20 drops. Therefore, one drop of dry sperm added to 10 cc. of sea water was considered to be a 1/200 dilution. Likewise, one drop of dry sperm added to 100 cc. of sea water would be a 1/2000 dilution. The minimum dilution of sperm used was 1/20 or one drop of dry sperm

to one cc. of sea water. The same volume of sperm suspension was used in all fertilization experiments. In all cases the sea water was filtered and used at the laboratory temperature immediately. After a preliminary run it was found that irradiation of spermatozoa to dilutions of 1/2000 or more could not tolerate exposures above 31,500 r. Therefore it was decided to use graded doses of exposure up to 31,500 r only.

The results of these experiments are shown in Table II.

The more dilute the spermatozoa the more radiosensitive, as measured by the fertilizing power of irradiated sperm and normal eggs. Further, the concentrated sperm at any level produced (qualitatively) more normal trochophores than did the diluted sperm, suggesting that even the genetic material may have been more adversely affected by irradiation in the diluted state.

Presuming that the genetic contributions of highly irradiated sperm might be of no significance (based on parthenogenetic stimulation of *Spisula* eggs by *Spisula* sperm irradiated to 264,600 r), it was conjectured that such sperm might be able to stimulate the eggs of the echinoderm, *Arbacia*, and cause parthenogenetic development. Interspecific crosses of this type have been accomplished among the amphibia (Rugh and Exner, 1940). This, however, could not be achieved in even a single *Arbacia* egg in many attempts with thousands of eggs, and concentrated and irradiated *Spisula* sperm. The physical structure of the *Spisula* sperm must be such as to be incompatible with the *Arbacia* egg cortex.

It was noted, however, that *Spisula* spermatozoa irradiated to 63,000 r or more and kept for some 24 hours, even in the refrigerator, gave evidence of secretion of a mucilaginous substance which caused them to be clumped together into long strings (see Fig. 3). Such secretion and clumping did not inactivate the spermatozoa as they were seen still to be vibratile as if stuck by their tail tips.

Spisula eggs

A single specimen of this clam will produce literally millions of eggs (Allen, 1951) all of which are in the germinal vesicle stage and each of which measures about 50 microns in diameter (see Fig. 1). If the eggs are properly washed at least twice, they can be used for as long as 8-10 hours at laboratory temperatures or up to 24 hours if kept in the refrigerator at 10 degrees C. Even after 48 hours at refrigeration about 40% of these eggs can be fertilized and will develop into normal larvae (trochophores).

In general the eggs of *Spisula* are more radiosensitive than are the spermatozoa, when measured by the ability to cleave and to develop into trochophores. An exposure of the eggs to 6,300 r is roughly equivalent to 18,900 r to the spermatozoa. Since motility cannot be a criterion of activity, the only clue of the intact egg to damage is its failure to be fertilized or to develop normally.

One might expect that the egg, with its abundant cytoplasm, would show damage more readily than would the spermatozoon which is almost devoid of cytoplasm. However a few eggs exposed to 252,000 r did cleave following fertilization with normal spermatozoa but none developed into any semblance of a larval trochophore. There is evidence of membrane and cytoplasmic damage in these eggs because most of them became ruptured and developed vesicular protrusions following fertilization with normal sperm. (Compare Figs. 1 and 2.)

X-irradiated eggs showed cleavage delay when fertilized by normal spermatozoa but the delay was more linear than when x-irradiated sperm were used to fertilize normal eggs. Also, the eggs did not tolerate the magnitude of doses used for the

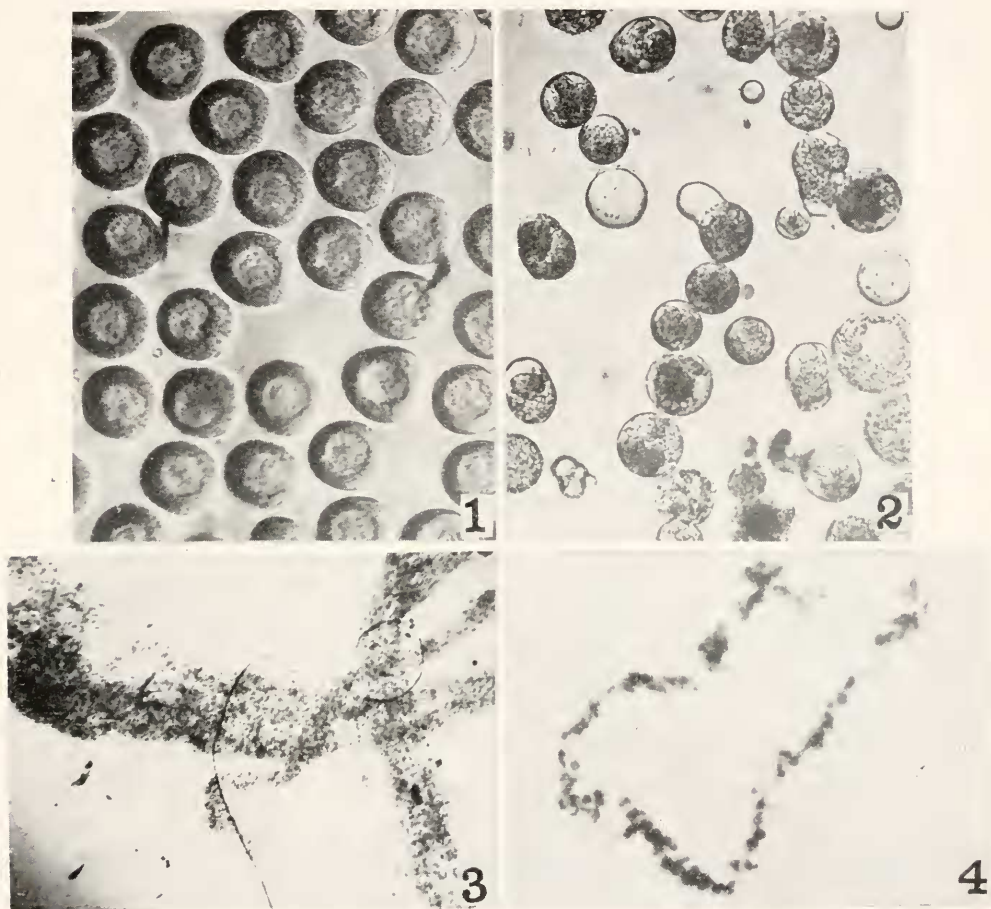


FIGURE 1. Normal eggs of *Spisula*, washed and ready for fertilization.

FIGURE 2. *Spisula* eggs exposed to 214,200 r x-rays and then fertilized by normal *Spisula* sperm, showing rupture and exovate masses. Some of the extruded masses and vesicles have rounded out to appear as miniature and deformed egg masses. Very few of these eggs ever cleaved, and none reached the trochophore stage.

FIGURE 3. *Spisula* sperm x-irradiated in cysteine which accentuated the secretion of a mucilaginous substance, causing the sperm to be aggregated into long strings.

FIGURE 4. Stringy aggregations of *Spisula* eggs x-irradiated in cysteine in sea water; reaction similar to that for spermatozoa (Fig. 3).

sperm. These data for the eggs include all eggs exposed but in the case of the spermatozoa it is impossible to determine what percentage of the invisible gametes are functional. At 3,150 r all eggs could be fertilized by normal sperm and would give rise to trochophores but at 31,500 r only some 70% of the eggs reached that

stage, even though there was 100% cleavage. The time of cleavage of 50% of x-irradiated eggs (fertilized by normal sperm) was as shown in Table III.

Spisula eggs exposed to higher levels of x-irradiation were able to cleave and develop. For instance, 90% of the eggs exposed to 94,500 r were fertilized and 40% became ciliated and motile trochophores. At 157,500 r there were also 90% blastulae and 10% trochophores and at 189,000 r there were very few cleavages and no trochophores developed.

Dilution of Spisula eggs had no appreciable effect on their radiosensitivity. The eggs were not irradiated while in the ovary because it was necessary to wash them twice in order to have them fertilizable at all. This meant that a concentration comparable to "dry sperm" (meaning gametes without any extra fluid) could not be obtained for the eggs. Nevertheless, eggs were concentrated after washing so that there was approximately a 1:1 ratio of eggs to sea water, and other eggs were suspended in sea water to give a 1:10 ratio. There was no appreciable difference in eggs irradiated in the two dilutions as measured by fertilizability and development. It is possible that even a 1:1 dilution (*i.e.*, the presence of any fluid) might be deleterious to eggs during x-irradiation.

TABLE III

	Time for 50% cleavage	Delay in minutes	Trochophores at 24 hours
Control	58"	0	100%
3,150 r	61"	3	
6,300 r	62"	4	60%
9,450 r	64"	6	
12,600 r	65"	7	15%
15,750 r	66"	8	
18,900 r	68"	10	5-10%
22,050 r	70"	12	
25,200 r	71"	13	10%
28,350 r	73"	15	
31,500 r	74"	16	5%

If eggs were irradiated and were then kept at 10° C. in the refrigerator for 24 hours and were brought to room temperature and fertilized, there was a higher percentage of cleavage and development to the trochophore stage than in eggs fertilized immediately after irradiation. It is not clear whether it was the delay or the refrigeration (or, possibly both factors) that seemed to be beneficial to the eggs. At 31,500 r some 40% ciliated trochophores appeared by 18 hours post-fertilization and at 63,000 r plus refrigeration some 50% trochophores developed. This may indicate some recovery on the part of the eggs under refrigeration, or it may indicate that toxic substances produced during irradiation in the medium in which the eggs were suspended had been dissipated.

There was some evidence of nuclear damage by exposure to 6,300 r since only about 60% of the eggs at this level reached the trochophore stage. The nuclear damage must have been most severe at 31,500 r (with 5% trochophore development) because as many as 40% trochophores again appeared following 94,500 r. Since further irradiation above 94,000 r did not improve trochophore production,

even though the change was still high, it may be presumed that cytoplasmic damage may have complicated the situation so that development could not proceed.

Spisula eggs exposed to various doses of x-irradiation were then submitted to normal spermatozoa of the echinoderm, *Arbacia*, pre-tested against eggs of *Arbacia*. At no level was a single irradiated *Spisula* egg fertilized by an *Arbacia* spermatozoon. At 264,600 r the *Spisula* eggs are ruptured by fertilization with normal *Spisula* sperm and develop ex-ovate masses, and disintegrate. If, however, such eggs are exposed to *Arbacia* sperm, these abnormal conditions do not occur, indicating that the *Arbacia* sperm are unable to penetrate the membrane and cortex even of these moribund eggs of *Spisula*.

In contrast with the irradiation of *Spisula* sperm, therefore, the egg is less tolerant and at the maximum exposure used (264,600 r) the egg membrane and cytoplasm were so damaged that fertilization was virtually impossible. The sperm, exposed to this dose, fertilized normal eggs to nearly 100%. With respect to the trochophores developing from irradiated sperm or irradiated eggs, there was no detectible difference.

Observations on Arbacia Gametes

Henshaw (1940) long since completed an exhaustive study of x-irradiation of *Arbacia* gametes. Nevertheless, as a parallel to the above *Spisula* experiments a few additional observations were made on *Arbacia* material.

Arbacia dry sperm x-irradiated to as much as 189,000 r were added to normal *Arbacia* egg suspensions and brought about normal cleavage in all eggs. Henshaw (1940) claimed obliteration of fertilization after exposure of the dry sperm to 300,000 r or more. However, 6 hours after such irradiation the "dry" *Arbacia* sperm, when diluted, were non-motile and could not be used for fertilization. This was not true for the similarly concentrated *Spisula* sperm which survived many hours post-irradiation, and even longer periods under refrigeration.

At 126,000 r about 50% of the embryos from irradiated sperm became blastulae and following 189,000 r and 252,000 r only occasional blastulae developed when the dry *Arbacia* sperm were irradiated, and used to fertilize normal eggs.

Even though motile after exposure to 189,000 r, the *Arbacia* sperm were never successful in fertilizing (activating) the normal eggs of *Spisula*. This interphyletic cross by irradiated sperm has not been attempted previously. It was thought that such sperm, with their genetic complement presumably damaged, might act as parthenogenetic agents and stimulate *Spisula* eggs at least to membrane elevation and cleavage. This, apparently, does not occur so that other incompatibilities must be present.

The cleavage delay following irradiation of *Arbacia* eggs (first reported by Henshaw, 1932 and Henshaw and Francis, 1933) was clearly substantiated. Further, *Arbacia* eggs exposed to x-rays at any level up to 126,000 r could not be fertilized by normal *Spisula* sperm. Again this suggests incompatibility apart from nuclear considerations since the irradiated nuclear material of the *Arbacia* egg was never reached by the normal *Spisula* sperm. Control situations, in which normal *Spisula* sperm were used with normal *Arbacia* eggs, never gave cleavage either. It was presumed that irradiated *Arbacia* eggs might have lost some of their resistance to foreign sperm. This was not borne out.

Arbacia plutei exposed to as little as 9,450 r very quickly aggregated into sticky clumps. This stickiness occurred soon after irradiation but did not last very long. It was probably due to a secretion from the irradiated plutei, for a similar stickiness has been reported for testicular chromosomes in other aquatic forms (Rugh, 1950). Plutei tolerated as much as 44,100 r with no more deleterious effects than a retardation in growth over a period of 7 days. The pluteus is therefore much less sensitive than is either gamete.

The original incentive for these studies was to determine whether chemical agents, previously demonstrated as having some protective value for mice against x-irradiation damage, might likewise have some protective effect on marine gametes and early embryos. The substances used were cysteine hydrochloride (Patt *et al.*, 1949, 1950) and another -SH compound known as B-mercaptoethylamine (Bacq *et al.*, 1951). Both substances have been reported to give protection to mice by injection before irradiation.

Cysteine

It was soon found that 0.5% cysteine was hypertonic and toxic to the eggs of *Spisula*; that a 0.1% solution allowed fertilization and cleavage but caused developmental abnormalities and that a 0.01% solution was toxic to developmental stages over extended periods but was not toxic for short periods to the gametes and early embryos of *Arbacia*. Fertilization could be accomplished to a normal degree in such a concentration of cysteine. Since exposure to cysteine was of rather short duration before, during and sometimes after irradiation, the concentration of 0.01% was used throughout the experiments.

Spisula eggs immersed in a 0.01% solution of cysteine in sea water (adjusted to pH 8.0) for as much as three hours before and during x-irradiation, had no effect whatever on fertilizability of the eggs, nor on the cleavage and early development. That is, both cleavage percentage and time were normal, similar to the controls which did not have the exposure to cysteine but did have the irradiation. Those eggs which were left in the solution in which they were irradiated, or were transferred to fresh cysteine, did not benefit by the presence of the cysteine with respect to developmental rate or degree when compared with the non-cysteine controls. In fact, if left more than a few hours no *Spisula* embryos reached the trochophore stage even though some of the non-irradiated controls did. Only when the cysteine concentration was reduced to 0.001% was there larval survival, but never to a degree of better than the non-cysteine controls. Exposure to cysteine before, during and after irradiation for a total of 1¼ hours did not affect later trochophore development. Eggs or embryos placed in previously irradiated cysteine were not thereby protected.

There was one bit of evidence of some cysteine effect. As stated above, when gametes or the larvae are x-irradiated to certain levels, they exude a sticky substance which causes them to aggregate (Figs. 3 and 4). This tendency is accentuated by the presence of cysteine. However, cysteine alone does not cause this and while x-irradiation does, the stickiness is much more extensive when living material is irradiated in the cysteine medium. Further, a lower exposure (6,300 r) of the eggs or plutei to x-rays causes the stickiness in cysteine while it does not do

so without the cysteine. The aggregations under x-rays alone are in small clumps at first. They appear as long and heavy strings in x-ray plus cysteine conditions.

While cysteine does not have any effect on cleavage time or percentage, and it does not in any way seem to protect the gametes or early embryo against x-irradiation, its presence does seem to cause a rounding out of the blastomeres of the two- and four-cell stages of both *Spisula* and *Arbacia*, stretching the intercellular connections so as to make them appear as though the blastomeres are almost unrelated. Many of the two-cell stages are almost separated. This is a situation one finds experimentally when such eggs are kept in a calcium-free or low-calcium medium.

B-Mercaptoethylamine (Bacq Solution)

This synthetic solution has a pH of 7.5 which is very close to that of normal sea water. It was adjusted to pH 8 before use. Bacq used 3 mg. per 30 gm. mouse, injected intraperitoneally, to give protection. This changed mortality of mice exposed to 700 r x-rays from 93% to 5%. When diluted to 0.1 mg. % in filtered sea water it was found to be lethal to *Arbacia plutei*; at 0.04 mg. % it was not toxic to the plutei but was toxic to the unfertilized eggs, causing a cortical wrinkling. The concentration which was not toxic, and which was finally used, was 0.01 mg. % at pH 8.0.

There was some slight statistical evidence that this solution allowed more eggs to develop and to develop further, following irradiation in the solution, than did the control situation of irradiation in sea water alone. There was also some slight evidence of acceleration of early cleavage. These were gross impressions which would have to be checked under highly controlled conditions of temperature and concentrations. Even then they would not be very significant, if confirmed, because the protection, if any, was so slight.

For instance, *Arbacia* eggs irradiated in the Bacq solutions did show a slightly higher percentage of development and the larvae developed farther than in the irradiated sea water controls. Also, *Spisula* eggs exposed to 18,900 r in the Bacq solutions survived to the trochophore stage better and in larger number than did their controls. But the degree of improvement in either case did not encourage an exhaustive statistical study since it was positive but not significant. One might say that if either solution showed any protection during irradiation it was the Bacq solution. In neither solution was development allowed to proceed normally very far.

SUMMARY AND CONCLUSIONS

1. The spermatozoa of the clam *Spisula* are more radioresistant than are its eggs.
2. The fertilizing power of *Spisula* sperm in the dry or concentrated state could not be affected by x-irradiation even to 264,000 r.
3. Increasing x-irradiation of *Spisula* spermatozoa caused increasing delay in cleavage time of normal eggs fertilized by such sperm, but the curve was not linear and did not exceed 15 minutes. This delay represents the time interval between the first and the second normal cleavages.

4. Trochophore production was at its lowest following 163,000 r x-irradiation of the spermatozoa, but with further x-irradiation trochophore production reached 95%. Such trochophores, while viable for a time, were not normal.

5. Dilution of *Spisula* spermatozoa increased their radiosensitivity as determined by the effect on subsequent embryonic development.

6. The demonstrated parthenogenetic stimulating ability of *Spisula* spermatozoa exposed to 189,000 r or more x-rays and used with *Spisula* eggs, could not be achieved when *Spisula* sperm were used with *Arbacia* eggs.

7. X-irradiated *Spisula* gametes exude a mucilaginous substance which causes them to aggregate (clump).

8. Very few *Spisula* eggs exposed to 189,000 r x-rays cleaved and none became trochophores. After 214,200 r there was evidence of cytoplasmic and membrane destruction.

9. Neither cysteine hydrochloride or B-mercaptoethylamine appeared to give any appreciable protection to *Spisula* gametes against x-irradiation as determined by the subsequent development of the embryo.

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